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Substituted coumarins as potent 5-lipoxygenase inhibitors

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Abstract—Leukotriene biosynthesis inhibitors have potential as therapeutic agents for asthma and inflammatory diseases. A novel series of substituted coumarin derivatives has been synthesized and the structure–activity relationship was evaluated with respect to their ability to inhibit the formation of leukotrienes via the human 5-lipoxygenase enzyme.

5-Lipoxygenase (5-LO) is a key enzyme in the biosynthesis of leukotrienes and catalyzes the initial steps in conversion of arachidonic acid to leukotrienes.[1,2] Inhibition of this enzyme may decrease leukotriene-mediated inflammatory responses and control disease states such as asthma.[3,4] Recent studies have implicated 5-LO activity[5–7] in a number of other diseases, including COPD, cancer, osteoporosis, and atherosclerosis, and several reviews have appeared recently highlighting the therapeutic potential of 5-LO inhibition.[8–14]

Many of the currently described inhibitors of 5-LO contain functional groups such as phenol, hydroxamate or *N*-hydroxyurea and act by a redox mechanism or by chelation of the active site iron. The multiple toxicities and difficulties encountered in developing redox inhibitors of 5-LO have led many research groups to strive to find competitive non-redox inhibitors of this enzyme.

We have previously introduced new classes of non-redox 5-LO inhibitors such as the pyridyl-substituted 2-cyanonaphthalene **L-739,010**[15] and the phenyl-substituted 2-cyanoquinoline **L-746,530**[16] (Fig. 1). While both inhibitors have excellent potency and pharmacokinetics in animal models, microsomal incubation studies revealed the formation of reactive intermediates at the dioxabicyclooctanyl moiety and precluded further development of these inhibitors.[17]

We therefore set out to identify replacements for the dioxabicyclooctanyl subunit as well as for the furyl substituent[18] in an effort to reduce the potential for toxicity. This work has culminated in the discovery of inhibitor **1** in which the 2-cyanoquinoline portion of **L-746,530** was replaced by a fluorophenyl-substituted coumarin and the dioxabicyclooctanyl moiety by a hexafluorocarbinol substituent.

From previous structure–activity relationship (SAR) studies we learned that the 2-cyanoquinoline moiety in **L-746,530** could be replaced by a 4-substituted coumarin.[19] This modification was a welcome observation

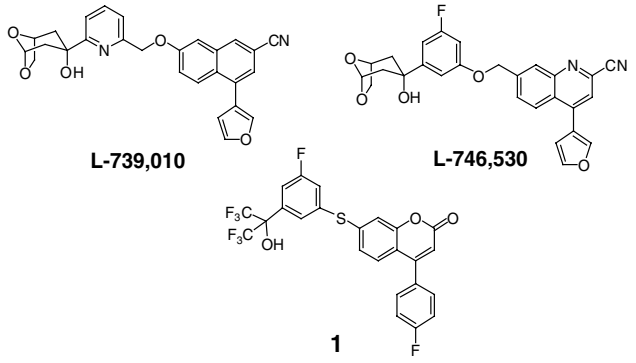

Figure 1.

Keywords: 5-Lipoxygenase; Leukotrienene inhibitor; Coumarin; Structure–activity relationship.
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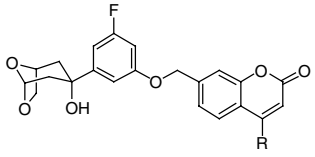
since, in theory, this would enable the preparation of coumarin prodrugs that could be delivered orally as water-soluble hydroxy acids that would cyclize in vivo to the active coumarin derivative (vide infra). All compounds prepared were evaluated for their potency to inhibit the oxidation of arachidonic acid by recombinant human 5-lipoxygenase (H5-LO),[20] the production of LTB_4 in calcium ionophore-stimulated human peripheral blood polymorphonuclear leukocytes (HPMN),[21] and the production of LTB_4 in calcium ionophore-stimulated human whole blood (HWB).[21] Although 10-fold less potent on H5-LO, no significant loss of inhibitory potency in HWB was observed by replacing the 2-cyanoquinoline in reference compound **L-746,530** with coumarin **2** (IC_{50} = 48 nM, Table 1, entry 2). The phenyl derivative **3** and the 4-fluorophenyl derivative **4**, however, were significantly less potent in the human whole blood assay with IC_{50}s of 440 and 1700 nM, respectively (Table 1, entries 3 and 4).

One of the most interesting avenues of SAR that was pursued, and that led to a wide range of inhibitors with acceptable potency, was derived from the preparation of different tertiary alcohols (Table 2).

In particular, the metabolically unstable dioxabicyclooctanyl subunit of **2** could be replaced by a variety of substituents (Table 2, entries 1–4). For example, the 2-thiazolyl ethyl derivative **5**[22] (racemic) (entry 1) and the 2-pyridinyl ethyl derivative **6** (racemic) (entry 2) are tolerated, while a small gain in potency in HWB (IC_{50} = 89 nM) can be achieved by replacing the heterocycle with an additional ethyl substituent to give **7** (entry 3). Although **7** exhibited excellent in vitro potency, it suffered from poor pharmacokinetics presumably due to metabolism at the geminal diethyl substituent. This problem could be alleviated by replacing the ethyl substituents with a geminal trifluoromethyl group to give **8** (entry 4). This bis(trifluoromethyl)carbinol moiety was found to be an excellent surrogate for the metabolically unstable dioxabicyclooctanyl alcohol and, therefore, was used exclusively in optimization studies.

In our earlier work, we demonstrated that the nature of the linkage (oxymethylene link vs thio link) between two aromatic moieties can have a profound effect on the potency of 5-LO inhibitors.[16] Therefore, it was pertinent to examine this structural modification in this series (Table 3). With the exception of the *p*-chloro-substituted inhibitor **9** (Table 3, entry 2), the furyl derivative **10**,

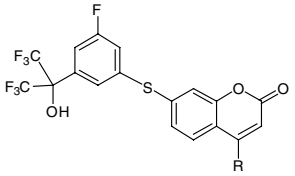
Table 1. SAR of dioxabicyclooctanyl-substituted coumarins



Entry	Compound	R	IC_{50}[a] (nM)		
			H5-LO[b]	HPMN[b]	HWB[b]
1	**L-746,530**		27 ± 10	2.3 ± 1.1	36 ± 18
2	**2**	3-Furyl	200 ± 70	3.0 ± 0.9	48 ± 17
3	**3**	Ph	300 ± 75	3.7 ± 0.6	440 ± 150
4	**4**	4-F–Ph	n.d.[c]	11.6 ± 0.1	1700[d]

[a] Mean ± SD.
[b] $n \geqslant 2$.
[c] Not determined.
[d] $n = 1$.

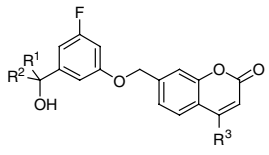
Table 3. SAR of hexafluorocarbinol-substituted coumarins



Entry	Compound	R	IC_{50}[a] (nM)		
			H5-LO[b]	HPMN[b]	HWB[b]
1	**1**	4-F–Ph	27 ± 16	0.5 ± 0.3	70 ± 24
2	**9**	4-Cl–Ph	180 ± 42	4.3 ± 3.7	400 ± 45
3	**10**	3-Furyl	9 ± 2.0	0.8 ± 0	52 ± 21
4	**11**	Ph	26 ± 19	0.5 ± 0.2	88 ± 21

[a] Mean ± SD.
[b] $n \geqslant 2$.

Table 2. SAR of carbinol-substituted coumarins



Entry	Compound	R^1	R^2	R^3	IC_{50}[a] (nM)		
					H5-LO[b]	HPMN[b]	HWB[b]
1	**5**	Et	Thia[c]	4-F–Ph	175 ± 20	2.7 ± 3.2	400 ± 10
2	**6**	Et	Pyr[c]	3-Furyl	175 ± 25	3.7 ± 1.7	360 ± 20
3	**7**	Et	Et	3-Furyl	15 ± 5.0	0.9 ± 0.6	89 ± 5.0
4	**8**	CF_3	CF_3	4-F–Ph	55 ± 4.0	1.9 ± 0.5	150 ± 75

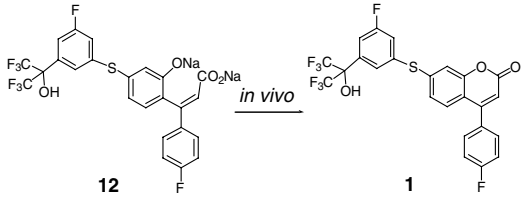
[a] Mean ± SD.
[b] $n \geqslant 2$.
[c] Thia = 2-thiazolyl, Pyr = 2-pyridinyl.

the phenyl derivative **11**, and the *p*-fluorophenyl derivative **1** (entries 3, 4, and 1) showed excellent inhibitory potency in all in vitro assays.

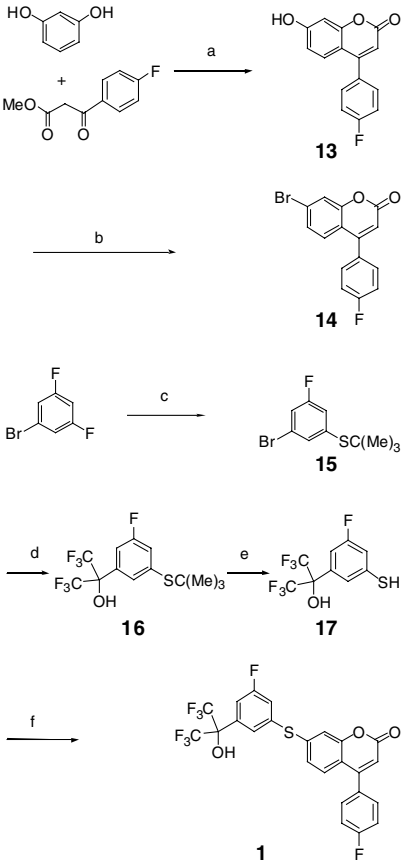
Bioavailability studies with **1** were performed by administering the water-soluble sodium salt of the corresponding hydroxy acid **12**[23] (Scheme 1).

This compound, when dosed orally in rats at 20 mg/kg in 0.5% methocel, was well absorbed and readily converted to active **1**.[24] Thus, upon dosing **12**, excellent bioavailability ($F = 100\%$) (0–24 h) and a maximum concentration (C_{max}) of 16–21 μM (1 and 4 h after dosing) were observed for **1**. Interestingly, oral administration of **1** as a suspension (20 mg/kg in 0.5% methocel) did not result in any absorption at all presumably due to low solubility. Therefore, **12** was used in all bioavailability studies. In squirrel monkeys, when **12** was dosed orally at 10 mg/kg in 0.5% methocel, bioavailability of 30% for **1** can be achieved with a C_{max} of 1.2 μM at 2 h. The inhibitory effect of **1** on the biosynthesis of LTB_4 in vivo was evaluated using the rat pleural cavity model following carrageenan-induced inflammation.[25] In this model, **1** showed activity with an ED_{50} of 1.6 mg/kg 2 h after oral dosing of **12**. The potency of **1** on: (1) the inhibition of urinary LTE_4 (an index of the systemic biosynthesis of peptidoleukotrienes) and (2) the ex vivo generation of LTB_4 in whole blood stimulated with calcium ionophore A23187 was measured in an anesthetized dog model.[26] **1** inhibited base-line urinary LTE_4 excretion, measured 5–7 h after the commencement of the infusion of **1** in 80% PEG200/water with an ED_{50} of 2.5 μg/kg/min. Compound **1** also inhibited the production of LTB_4 in dog whole blood ex vivo with an ED_{50} of 2.5 μg/kg/min. Microsomal metabolism studies with radiolabeled **1**[27] showed that there was no formation of radioactive metabolites covalently bound to protein, a problem we had encountered with our previous inhibitors **L-737,010** and **L-746,530**.[17]

The synthesis of **1** was accomplished (Scheme 2) in a convergent manner by the preparation and coupling of two advanced intermediates, the aromatic thiol **17** and the bromocoumarin **14**. Preparation of bromide **14** commenced with a von Pechmann condensation[28] of resorcinol and methyl 4-fluorobenzoylacetate under acidic conditions to provide phenol **13** in 79% yield. Treatment of **13** with bromine (neat) and triphenylphosphine at 280 °C furnished **14** in 62% isolated yield.[29] Thiol **17** was prepared in a four-step sequence starting with commercially available 1-bromo-3,5-difluorobenzene and 2-methyl-2-propanethiol.[30] The coupling product **15**, obtained in 78% yield, was then engaged in a Grignard reaction with liquified hexafluoroacetone to yield **16** in 72% yield. Deprotection of **16** with mercury(II) acetate and sodium sulfide[31] gave thiol **17** in 56% isolated yield. Final coupling of **17** and **14** with potassium carbonate in 1-methyl-2-pyrrolidinone (NMP) furnished **1** in 66% yield as an off-white solid.



Scheme 1.



Scheme 2. Reagents and conditions: (a) HCl, MeOH, 0 °C to rt, 15 h (79%); (b) Br_2, PPh_3, 280 °C, 1 h (62%); (c) Me_3CSH, NaH, DMF, −10 °C to 0 °C, 24 h (78%); (d) Mg, CF_3COCF_3, THF, 0 °C, 1.25 h (72%); (e) 1—$Hg(OAc)_2$, PhOMe, TFA, DMF, 60 °C, 3.5 h; 2—$Na_2S{\cdot}7H_2O$, rt, 0.25 h (56%); (f) K_2CO_3, NMP, 100 °C, 1 h (66%).

The results of the present study demonstrate that the structures of **L-739,010** and **L-746,530** can be modified to yield 5-LO inhibitors with comparable in vitro potency. With the bis(trifluoromethyl)carbinol moiety we have identified an alternative motif for the dioxabicyclooctanyl ring system. In addition, we have replaced the furyl-substituted 2-cyanoquinoline with a 4-fluorophenyl coumarin and the optimized 5-LO inhibitor **1** has excellent pharmacokinetics in rats when dosed as the prodrug **12**.[32]

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23. Compound **12** was prepared by treating **1** with 1M NaOH (2 equiv) in THF (65 °C, 2 h). The solvent was evaporated, the residue was dissolved in de-ionized water, and the solution was lyophilized to give **12** as a light yellow powder.
24. Compound **12** acts as the prodrug of **1**. Compound **12** did not show activity in primary in vitro assays: H5-LO IC_{50}: 30% inhib. at 60,000 nM, HPMN IC_{50} = 17,000 nM, HWB IC_{50} n.d.
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